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# Acyclovir, Ganciclovir, and Zidovudine Transfer into Rat Milk

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**Treatment with antiviral agents that accumulate in breast milk may offer a novel approach to reduce the rates of vertical transmission of important viruses and the risk of clinical illness in suckling neonates. The present study evaluated the extent and mechanism of transfer of three antiviral nucleoside analogues into milk in a lactating rat model system. Acyclovir (0.26 mg/h), ganciclovir (0.13 mg/h), and zidovudine (0.5 mg/h) were each infused to steady-state concentrations in six rats 15 to 16 days postpartum. The observed ratios of the concentrations in milk to the concentrations in serum (observed milk-to-serum ratio), calculated from the ratio of the steady-state concentration in serum to the steady-state concentration in milk, determined the extent of drug transfer into milk. To identify the mechanism of transfer into milk, the observed milk-to-serum ratio was compared to a predicted milk-to-serum ratio estimated from an in vitro passive diffusion model of transfer of each drug into milk. High-pressure liquid chromatography methods determined milk and serum drug concentrations. Mean ± standard deviation observed milk-to-serum ratios for acyclovir, ganciclovir, and zidovudine were 5.1 ± 1.4, 1.6 ± 0.33, and 1.0 ± 0.29, respectively, compared with their corresponding predicted ratios of 1.1, 0.85, and 0.71. These results suggest that acyclovir accumulates in milk due to active transport mechanisms, while passive diffusion processes govern the transfer of both ganciclovir and zidovudine into milk. The presence of all three antiviral drugs in milk and the potential for active drug transfer into milk warrants further investigation of the accumulation of other antiviral drugs in milk and their therapeutic benefits in reducing the vertical transmission of viruses and clinical sequelae in the breast-feeding infant.**

The nucleoside analogues acyclovir (ACV), ganciclovir (GCV), and zidovudine (AZT) are commonly used to treat important viral diseases (herpesvirus infections, cytomegalovirus infections, and AIDS). Breast-feeding places a suckling infant at a significant risk of exposure to most viral infections since vertical transmission may occur via the breast milk. The transmission of viruses such as human T-cell lymphotropic virus type 1 and human immunodeficiency virus type 1 during breast-feeding (1, 13, 35, 50) may lead to significant illness in a suckling infant. For mothers who have no alternative to breast-feeding (e.g., mothers in developing countries), current strategies designed to reduce the rates of viral transmission from the mother to the infant throughout the lactation period have limited benefit. Treatment of the mother during breast-feeding with antiviral agents that accumulate in breast milk may offer a novel approach to the lowering of viral transmission rates. In addition to lowering the maternal viral load, this strategy has the potential to directly and/or indirectly reduce viral titers in breast milk, to reduce the levels of exposure of the neonate to virus during breast-feeding, and to provide a therapeutic dose to the neonate. Such characteristics may significantly reduce the risk of clinical disease in suckling neonates.

At present, few studies have evaluated the concentrations of antiviral drugs in milk and the mechanisms of their transfer into milk. The literature suggests that some drugs may accumulate in milk at concentrations significantly higher than the concentrations in maternal serum (16, 25, 36, 37). Several studies have reported on the accumulation of the guanosine analogue ACV in human milk at concentrations approximately threefold greater than the concentrations in maternal serum (2, 12, 27, 32, 43). The thymidine analogue AZT achieves ratios of the concentration in milk to the concentration in serum (M/S ratios) of 1.2 to 1.8 (5). The M/S ratios reported in the previous studies were obtained from the measurement of concentrations in milk and concentrations in serum at one time point, but time-dependent M/S ratios may lead to inaccurate estimations of M/S ratios for these drugs (48). Whether these drugs accumulate significantly in milk is not exactly known.

Clinical studies designed to evaluate the distributional kinetics of drugs in milk during lactation are difficult to perform with breast-feeding women. Few well-controlled and properly executed pharmacokinetic studies defining the M/S ratios of drugs exist, and fewer yet are available for antiviral agents. Previous studies have shown that the lactating rat model reliably predicts drug transport characteristics in human breast milk (14–17, 23, 30, 31, 37). Consequently, this animal model system may provide information about the distribution characteristics of important antiviral drugs in milk and the therapeutic benefit of antiviral drug accumulation in milk.

The purpose of this study was to evaluate the extent and mechanism of transfer of three model antiviral nucleoside analogues, ACV, GCV, and AZT, into milk in a lactating rat model. These antiviral agents were chosen due to evidence in the literature of their potential for accumulation in human milk and/or their common physicochemical characteristics (nucleoside analogues) and potential to have similar transport mechanisms. Data from these studies may help further validate the lactating rat model as an effective in vivo screening test for

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the identification of the mechanism of transfer of antiviral agents into milk and their potential therapeutic effects.

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## MATERIALS AND METHODS

**Chemicals.** ACV, AZT, and β-hydroxypropyltheophylline were purchased from Sigma Chemical Co. (St. Louis, Mo.). ACV sodium for injection (manufactured by Abbott Laboratories, Chicago, Ill.) was obtained from VHA Inc. GCV was a kind gift from Hoffmann-La Roche, Inc. (Nutley, N.J.). All other reagents were analytical grade. All solvents were high-pressure liquid chromatography (HPLC) grade.

**Animals.** Lactating female Sprague-Dawley rats were obtained from Harlan Laboratories (Indianapolis, Ind.) and were allowed an acclimatization period. The University of Kentucky Institutional Animal Care and Use Committee approved all animal care and treatment procedures before initiation of the studies.

**In vivo determination of M/S ratios. (i) Intravenous infusion to steady-state concentrations.** At 14 or 15 days postpartum, 24 h before commencement of the study, the right jugular and left femoral veins of lactating dams were surgically cannulated while the rats were under ketamine (100 mg/kg of body weight) and xylazine (6 mg/kg) anesthesia (intraperitoneal injection). On the day of the study the pups were removed from the dams 2 h prior to the infusion. ACV (0.26 mg/h for 8 h), GCV (0.13 mg/h for 8 h), and AZT (0.5 mg/h for 12 h) were infused via the femoral vein catheter until steady-state concentrations were obtained in a total of six rats for each drug. A small pilot study determined that ACV and GCV reached steady-state levels in blood by 6 h and that AZT reached steady-state levels in blood by 8 h. In this pilot study, the concentrations of each drug in serum were measured at 2, 4, 6, 8, 10, and 12 h. The time point at which serum drug concentrations remained similar to the concentrations measured at later time points established the time required to reach steady state. Furthermore, manual expression of milk from the rats required anesthesia, which precludes the collection of multiple milk samples to ensure steady-state levels of drug in milk. Rather, the drug concentrations in milk were assumed to closely parallel the drug concentrations in serum. An extension of the infusion period beyond the time required to reach steady-state levels of drug in serum accounted for a possible delay in the distribution kinetics in milk relative to those in serum. It was assumed that the duration of the infusion period was sufficient to achieve steady-state concentrations of drug in milk. Consequently, serial blood samples (~300 μl) were drawn via the jugular vein catheter at 5, 6, 7, and 8 h for ACV and GCV and at 6, 8, 10, and 12 h for AZT. Immediately following collection of the last blood sample (i.e., at the end of the infusion period), a single milk sample (~200 μl) was collected by manual expression of milk while the dams were under light ketamine anesthesia. All samples were stored frozen (−20°C) until analysis.

**(ii) M/S ratios$_{Observed}$.** Observed M/S ratios (M/S ratios$_{Observed}$) for the parent drug were determined from the ratio of the steady-state concentration in milk ($C_{ss,\ milk}$) to the steady-state concentration in serum ($C_{ss,\ serum}$) for the respective antiviral agent, as follows:

$$\text{M/S ratio}_{\text{Observed}} = \frac{C_{ss,\ milk}}{C_{ss,\ serum}} \quad (1)$$

**In vitro determination of M/S ratios$_{Predicted}$.** M/S ratios for hydrophilic drugs whose transfer into milk is governed by passive diffusion may be predicted from an in vitro passive diffusion model (14, 15):

$$\text{M/S ratio}_{\text{Predicted}} = \frac{f_s^u f_s}{f_m^u f_m\ (\text{S/W})} \quad (2)$$

where $f$ is the free fraction of drug in serum or milk, $f^u$ is the unionized fraction of drug in serum or milk, S/W is the ratio of the drug concentration in skim milk to the drug concentration in whole milk, and the subscripts $s$ and $m$ represent serum and milk, respectively.

The S/W ratio, a measure of the milk fat partitioning of drug, was determined following HPLC analysis of the drug concentrations in blank rat whole milk and skim milk into which drug had been spiked. The unionized fraction in serum and skim milk, a measure of the pH partitioning of drug between serum and milk, was calculated from the $pK_a$ of each drug and from the pHs of serum (pH 7.46) and milk (pH 6.67) (D. E. Burgio and P. J. McNamara, unpublished data). The free fraction in serum and skim milk, a measure of the extent of protein binding to serum and milk proteins, respectively, was determined by ultrafiltration. Briefly, blank rat serum (500 μl) and skim milk (300 μl) were spiked with two different concentrations of drug (1 and 5 μg/ml for ACV and AZT; 2 and 5 μg/ml for GCV) at room temperature. Samples were loaded onto a Centrifree Micropartition system (Millipore Corp., Bedford, Mass.) containing a YM10 ultrafiltration membrane (Millipore Corp.) and centrifuged at room temperature (Sorvall RT 6000D; Kendro Laboratory Products, Newtown, Conn.) under conditions previously optimized to achieve an ultrafiltrate volume of <20% of the initial sample volume in <5 min of centrifugation time. As well, complete recovery of ACV, GCV, and AZT spiked into water following ultrafiltration verified the absence of drug binding to the ultrafiltration membrane and device.

**HPLC analysis of ACV, GCV, and AZT concentrations in rat serum and milk. (i) HPLC analysis of ACV concentrations.** ACV concentrations in rat serum (100 μl) and milk (50 μl diluted to 100 μl with double-distilled water) samples were determined by a modified method of Hedaya and Sawchuk (21). Briefly, 25 μl of internal standard (GCV at 20 μg/ml in double-distilled water) was added to all unknown and calibration samples in 1.5-ml microcentrifuge tubes. One milliliter of acetonitrile was added to all tubes, and the contents were vortex mixed for 10 min to precipitate the proteins. The samples were centrifuged at 14,000 rpm in an Eppendorf centrifuge (model 5415; Brinkman Instruments Co., Westbury, N.Y.), and the supernatant was transferred to screw-top culture tubes (16 by 125 mm) containing 0.3 ml of an aqueous solution of 2% (wt/vol) monobasic ammonium phosphate. Five milliliters of diethyl ether was added, the contents were vortex mixed for 10 min, and the tubes were centrifuged at 3,200 rpm for 10 min in a Sorvall RT 600D centrifuge. The organic layer was discarded; and an additional 5 ml diethyl ether was added, the contents were vortex mixed for 5 min, and the tubes were centrifuged. The organic layer was again discarded, and a 50-μl aliquot of the aqueous portion was injected onto a Shimadzu HPLC system with UV detection. Chromatographic separation was achieved on a Supelcosil $C_{18}$ reversed-phase column (15 cm by 4.6 mm; LC-18; Supelco, Inc., Bellefonte, Pa.) and was monitored at 250 nm. The mobile phase was pumped at an isocratic flow rate of 1.0 ml $min^{-1}$ and consisted of 1% acetonitrile and 99% 10 mM monobasic ammonium phosphate (pH 6.8 with ammonium hydroxide).

**(ii) HPLC analysis of GCV concentrations.** HPLC analysis of GCV concentrations was similar to that of ACV concentrations, except that ACV (20 μg/ml in double-distilled water) was used as the internal standard, chromatographic separation was achieved with a Lichrosorb 5 RP18 reversed-phase column (12.5 cm by 4 mm; Phenomenox, Torrance, Calif.) monitored at 250 nm, and the mobile phase consisted of 0.5% acetonitrile and 99.5% 10 mM monobasic ammonium phosphate (pH 6.8).

**(iii) HPLC analysis of AZT concentrations.** AZT concentrations in rat serum (100 μl) and milk (50 μl diluted to 100 μl with double-distilled water) samples were analyzed by HPLC by a modification of the method of Hedaya and Sawchuk (22). Briefly, 20 μl of the internal standard (β-hydroxypropyltheophylline at 7.5 μg/ml in double-distilled water) was added to all unknown and calibration samples in 1.5-ml microcentrifuge tubes. One milliliter of acetonitrile was added, the contents were vortex mixed for 10 min, and the tubes were centrifuged at 14,000 rpm in an Eppendorf centrifuge (model 5415; Brinkman Instruments Co.) to precipitate the proteins. The supernatant was evaporated to dryness under a stream of nitrogen, and the residue was reconstituted in 150 μl of the mobile phase. A 50-μl aliquot was injected onto a Shimadzu HPLC system with UV detection. Chromatographic separation was achieved on a Lichrosorb 5 RP18 reversed-phase column and monitored at 266 nm. The mobile phase was pumped at an isocratic flow rate of 1.0 ml $min^{-1}$ and consisted of 11% acetonitrile and 89% 10 mM monobasic ammonium phosphate (pH 6.8).

Linearity was established by plotting the ratios of the peak height of the drug to the peak height of the appropriate internal standard against the calibration standard concentrations. Linear calibration curves with $r^2$ values >0.998 were produced. Unknown sample concentrations were determined from interpolation of the calibration curve. Interassay and intra-assay accuracies and precision coefficients of variation were <10%. Quality control samples with low, medium, and high concentrations of the drugs were analyzed in duplicate for each analysis run for use as acceptance criteria for the analysis.

**Statistical analysis.** To determine whether active transport mechanisms governed ACV, GCV, or AZT transfer into milk, a modification of the statistical analysis of Gerk et al. (16) was used. A 95% confidence interval was determined for the M/S ratio$_{Observed}$ for each antiviral drug and compared with values that were 50 and 200% of the M/S ratios$_{Predicted}$. If the upper limit of the 95% confidence interval for the M/S ratio$_{Observed}$ exceeded 200% of the M/S ratio$_{Predicted}$, an active transport process for antiviral transfer into milk was supported.

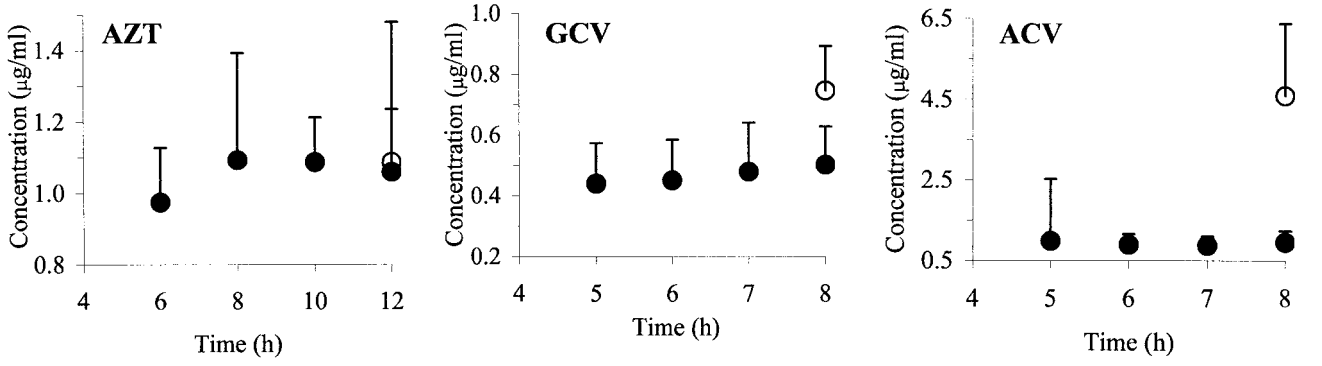


FIG. 1. Average ± standard deviation concentrations of AZT, GCV, and ACV in serum (closed symbols) and milk (open symbols) as a function of time to steady-state concentrations in lactating Sprague-Dawley rats ($n$ = 6) 15 to 16 days postpartum. A single milk sample was collected immediately following collection of the last serum sample.

## RESULTS

Continuous intravenous infusion produced steady-state concentrations in serum by 5 h for ACV and GCV and by 8 h for AZT (Fig. 1). M/S ratios were determined from the average of the concentrations in serum at 6, 7, and 8 h and the concentration in milk at 8 h for ACV and GCV and from the average of concentrations in serum at 8, 10, and 12 h and the concentration in milk at 12 h for AZT. Table 1 reports the individual and mean ± standard deviation M/S ratios$_{Observed}$ for each drug. M/S ratios$_{Observed}$ for ACV were higher and more variable (mean, 5.07) than that for either GCV or AZT, whose M/S ratios$_{Observed}$ more closely approximated unity.

TABLE 1. M/S ratios$_{Observed}$ and M/S ratios$_{Predicted}$ for ACV, GCV, and AZT in lactating Sprague-Dawley rats[a]

| M/S ratio and property or rat no. | M/S ratio | | |
|---|---|---|---|
| | ACV | GCV | AZT |
| M/S ratio$_{Predicted}$ | | | |
| p$K_a$ | 9.25[b] | 9.4[c] | 9.68[d] |
| $f_{u,serum}$ | ~1 | ~1 | ~1 |
| $f_{u,milk}$ | ~1 | ~1 | ~1 |
| $f_{serum}$ | 0.67 | 0.83 | 0.70 |
| $f_{milk}$ | 0.68 | 0.98 | 0.88 |
| S/W ratio | 0.93 | 1.01 | 1.11 |
| M/S ratio$_{Predicted}$ | 1.07 | 0.85 | 0.71 |
| 200% M/S ratio$_{Predicted}$ | 2.14 | 1.7 | 1.42 |
| 50% M/S ratio$_{Predicted}$ | 0.54 | 0.43 | 0.36 |
| (M/S ratio$_{Observed}$)/(M/S ratio$_{Predicted}$) | 4.74 | 1.92 | 1.40 |
| M/S ratio$_{Observed}$ | | | |
| 1 | 5.51 | 1.50 | 0.91 |
| 2 | 4.22 | 1.67 | 0.77 |
| 3 | 7.14 | 1.42 | 1.47 |
| 4 | 3.37 | 1.23 | 1.18 |
| 5 | 4.40 | 2.15 | 0.66 |
| 6 | 5.79 | 1.85 | 0.99 |
| Mean | 5.07 | 1.64 | 1.00 |
| SD | 1.35 | 0.33 | 0.29 |
| Upper 95% CI[e] | 6.48 | 1.98 | 1.30 |
| Lower 95% CI | 3.66 | 1.29 | 0.69 |

[a] The M/S ratios were determined 15 to 16 days postpartum.
[b] From reference 9.
[c] From reference 4.
[d] From reference 6.
[e] CI, confidence interval.

Table 1 reports the calculated fractions unionized ($f$), the calculated fractions unbound ($f'$), and the S/W ratio for each drug. These values were substituted into equation 2 to estimate the M/S ratio$_{Predicted}$ for each drug (Table 1). The fraction of unionized drug approximated 1 for each drug since their p$K_a$ values (acidic group) exceeded the pHs of lactating rat serum (pH 7.46) and milk (pH 6.67) by nearly 2 orders of magnitude. The free fraction of ACV determined by ultrafiltration was similar for samples spiked with 1 and 5 μg/ml (data not shown), and the fractions of drug binding in serum and skim milk were comparable. The level of serum protein binding of ACV (0.33) agrees reasonably well with the low level of binding reported in the literature for various species (9). Similarly, the free fractions of GCV and AZT in serum and milk were similar for samples spiked with 1 or 2 μg/ml and 5 μg/ml (data not shown). However, less binding was observed in skim milk than in serum for each drug (Table 1). The literature reports protein binding values of 21 to 27% for AZT in rats, which agrees reasonably well with the level of protein binding estimated by ultrafiltration (30%) in the present study (18). The fat partitioning of each drug was minimal, with ACV showing the greatest degree of partitioning into fat (S/W ratio, 0.93). All three drugs are relatively hydrophilic in nature, and therefore, significant distribution of the drugs into milk fat was not expected.

The M/S ratio$_{Observed}$ for ACV was ~3.5- to 7-fold greater (mean, 5.07) than the M/S ratio$_{Predicted}$ (Table 1), and the 95% confidence intervals around the M/S ratio$_{Observed}$ (Table 1) did not overlap the 50 to 200% range of the M/S ratio$_{Predicted}$ (Table 1). GCV and AZT demonstrated more modest M/S ratios$_{Observed}$ compared to the M/S ratios$_{Predicted}$, with M/S ratios$_{Observed}$ to M/S ratios$_{Predicted}$ not exceeding twofold (Table 1). Additionally, the 95% confidence intervals around the M/S ratio$_{Observed}$ (Table 1) for both GCV and AZT did overlap the 50 to 200% range of the M/S ratio$_{Predicted}$ (Table 1).

## DISCUSSION

The M/S ratios of three antiviral drugs, ACV, GCV, and AZT, were studied in a lactating rat model and compared to the data in

the literature available for humans. M/S ratios$_{Observed}$ of 5, 1.6, and 1 for ACV, GCV, and AZT, respectively, are consistent with reports in the literature of significant accumulation of ACV in human breast milk (2, 12, 27, 43) and a reported absence of accumulation of GCV and AZT in human breast milk (5). These data suggest that ACV, but not GCV and AZT, accumulates to significant levels in human breast milk. These data also suggest that the lactating rat model may serve as an effective screening tool to predict the distribution characteristics of antiviral drugs in the milk of lactating women.

The M/S ratio is an index of the extent of drug transfer into milk relative to the levels of drug in plasma. Previous work had shown that the passive transfer of hydrophilic weak organic acids and bases across the lactating mammary epithelium generally produces M/S ratios$_{Observed}$ $\leq$1 (14, 15). Protein binding, the degree of ionization, and the extent of fat partitioning govern the passive transfer of drugs across the lactating mammary epithelium (14). On the basis of these determinants, an in vitro diffusion model predicts M/S ratios $\leq$1 for many hydrophilic drugs whose transfer into milk is mediated by passive diffusion (15). When M/S ratios$_{Observed}$ appreciably exceed unity, active drug transport mechanisms at the lactating mammary epithelium may explain the significant accumulation of drug in milk. Allowing for normal physiological and interindividual variabilities, M/S ratios$_{Observed}$ must exceed M/S ratios$_{Predicted}$ by at least twofold to provide suggestive evidence of active transport-mediated drug accumulation in milk (unpublished data).

The fivefold difference between the M/S ratio$_{Observed}$ and the M/S ratio$_{Predicted}$ for ACV in the lactating rat model suggests that active transport principally mediates ACV transfer into rat milk. Several studies with lactating women have inferred the active transport of ACV into human breast milk based upon M/S ratios$_{Observed}$ of ~3.3 (the average from the literature) after determination of the concentrations at a single time point (2, 12, 27, 32, 43). Observations from the lactating rat model suggest that ACV accumulates to significant levels in human breast milk and that the lactating rat model may reliably predict the mechanisms of the distribution of ACV in human milk.

The literature provided no information concerning GCV levels in human breast milk. A difference in the M/S ratio$_{Observed}$ versus the M/S ratio$_{Predicted}$ of less than twofold for GCV in the lactating rat model suggests that passive processes govern the transfer of GCV into milk. Similarly, a 1.4-fold difference between the M/S ratio$_{Observed}$ and the M/S ratio$_{Predicted}$ of AZT in the lactating rat model implies that the transfer of AZT across the lactating mammary gland involves only passive diffusion processes. These results compare well with those of Greene et al. (20), who reported nearly equivalent concentrations in maternal serum and milk (M/S ratio, 1.14) in rats at 15 days postpartum based upon calculation of an estimated area under the concentration-time curve for drug in milk and serum consequent to a chronic oral dosing regimen. Data from the lactating rat model agree with M/S ratio estimates of 1.2 to 1.8 for AZT reported in lactating women (5). Again, the lactating rat model successfully predicted the passive transfer of GCV and AZT into human milk.

Although the present study provides suggestive evidence for the active transport of ACV into breast milk, Bork et al. (3), using an in vitro two-chamber system, concluded that passive transfer mechanisms are sufficient to explain the high M/S ratios of ACV. Those investigators suggest that the slightly lipophilic properties of ACV result in its partitioning into milk fat and enhanced concentrations in breast milk relative to those in plasma (3). As well, they concluded that differences in protein binding characteristics do not significantly contribute to the accumulation of ACV in milk (3). In the present study, the S/W ratio for rats approximated unity (Table 1), suggesting a negligible degree of ACV fat partitioning in milk. As well, the levels of protein binding between milk and serum were nearly identical (Table 1). Before the conclusions of Bork et al. (3) are accepted, a comparison of the two-chamber device with an equilibrium dialysis system to control for such factors as temperature fluctuations, chamber fluid fluctuations, consistency of the chamber environment, mixing in the chamber, and biological and chemical stabilities is necessary to ensure the validity of their observations.

Differences in the mechanisms of the distributions of the nucleoside analogues ACV, GCV, and AZT in milk are surprising because the literature provides evidence of their protein carrier-mediated transport across cell membranes. Studies have shown that ACV, GCV, and AZT are substrates for nucleoside and/or nucleobase transport systems (11, 28, 29, 38, 49, 51). As well, rat OAT1 (rOAT1) (47) and a probenecid-sensitive carrier-mediated transport system at the blood-brain barrier mediate both ACV and AZT transport across cell membranes (24, 44). Interestingly, probenecid is actively transported into rat milk, with an M/S ratio$_{Observed}$ fourfold greater than the M/S ratio$_{Predicted}$ (17). Probenecid is a substrate for multidrug resistance-associated protein, and the uric acid transporter (39) and is an inhibitor of both OAT1 and OAT3 (26, 41, 46). Gerk et al. (17) demonstrated rOCT1 and rOCT3 mRNA expression but not rOAT1, rOAT2, and rOAT3 mRNA expression in the lactating rat mammary gland. This evidence suggests that rOAT1 is not involved in the active transport of ACV into rat milk. Dhillon et al. (10) reported the presence of human OCT1 (hOCT1) in mammary epithelial cells isolated from human breast milk. Additionally, Kwok et al. (B. Kwok, V. Cook, A. Tropea, and S. Ito, Clin. Pharmacol. Ther. **69:**P19, 2001) reported that hOCTN1 and hOCTN2 are expressed in a mammary myoepithelial cell line (HMEC) and in mammary tissue and reported on their function in cationic drug transport in the human mammary gland. However, the transport of ACV, GCV, and AZT by these organic cation transport systems has not been documented. Failure to observe active transport of GCV and AZT into milk may suggest that a separate, yet unidentified, mechanism mediates ACV transport into milk. Alternatively, these antiviral analogues may share similar transport mechanisms, but differences in their affinities for their transporters may obscure an active transport process for GCV and AZT by the lactating mammary epithelium in vivo.

The characteristics of the distribution of antiviral agents in milk are of paramount importance due to the risk associated with the vertical transmission of viruses (e.g., human immunodeficiency virus type 1) with breast-feeding (8, 19, 34). Antiviral drugs that accumulate to significant levels in milk may have a greater therapeutic benefit in reducing viral titers in milk and/or providing a higher antiviral dose to the nursing infant (7, 33, 45). A study with mice reported that AZT concentrated in milk at levels up to 5.5-fold greater than the levels in ma-

ternal serum (40). AZT treatment failed to significantly alter the titers of Moloney murine leukemia virus in milk or prevent transmission of the virus to the neonate, but treatment did reduce the viral loads in the infected offspring and suppressed the development of disease in all neonatal mice (42). Since the accumulation of significant levels of drug in breast milk may have a greater benefit in reducing the rates of vertical virus transmission, ACV may function as a model substrate for characterization of the kinetics of active transport of other antiviral drugs into milk. Such knowledge may allow the implementation of strategies that enhance antiviral drug penetration into milk and that exert a greater therapeutic effect. Consequently, chronic maternal dosing with antiviral drugs that accumulate to significant levels in breast milk may pose a public health strategy for lowering the risk of vertical transmission of viruses to the breast-fed infant.

In conclusion, M/S ratios$_{Observed}$ versus M/S ratios$_{Predicted}$ suggest that ACV is actively transported into rat milk but that passive diffusion processes govern GCV and AZT transfer into milk. The lactating rat model may provide an excellent screening tool for assessment of antiviral drug distribution into human milk and the potential therapeutic benefits of the presence of drugs in milk before clinical trials are conducted with humans. The presence of all three antiviral drugs in milk warrants further investigation into the accumulation of other antiviral drugs in milk and their therapeutic benefits in reducing the vertical transmission of viruses and clinical sequelae in the breast-feeding infant.

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